

## Enhancer Traps in the *Drosophila* Bithorax Complex Mark Parasegmental Domains

Kimberly McCall,\* Michael B. O'Connor<sup>†</sup> and Welcome Bender\*

\*Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115,  
and <sup>†</sup>Department of Biochemistry and Molecular Biology, University of California at Irvine, Irvine, California 92717

Manuscript received April 12, 1994

Accepted for publication July 6, 1994

### ABSTRACT

Eight *P* elements carrying a  $\beta$ -galactosidase (*lacZ*) reporter have been mapped to sites within the *Drosophila* bithorax complex. The bithorax complex contains three homeotic genes, and at least nine regulatory regions which control their expression in successive parasegments of the fly. The enhancer traps inserted at the promoter of one of the genes, *Ultrabithorax*, express *lacZ* in patterns which mimic the *Ultrabithorax* protein pattern. Enhancer traps in the regulatory regions do not mimic the endogenous genes, but express *lacZ* globally in the relevant parasegments. Some *P* elements carry large DNA fragments upstream of the *lacZ* promoter but internal to the *P* element. In cases where these internal sequences specify a *lacZ* pattern, that pattern is generally suppressed when the element is inserted in the bithorax complex. In embryos mutant for genes of the *Polycomb* group, the *lacZ* expression from the enhancer traps spreads to all segments. Thus, the enhancer traps reveal parasegmental domains that are maintained by *Polycomb*-mediated repression. Such domains may be realized by parasegmental differences in chromatin structure.

THE identity of each segment of *Drosophila* is determined by the homeotic genes that are expressed in that region. In the fly these homeotic genes are found in two clusters: the Antennapedia complex and the bithorax complex (BX-C) (LEWIS 1978; KAUFMAN *et al.* 1980). The three genes of the BX-C (*Ubx*, *abdA* and *AbdB*) determine the segment identities of the third thoracic through ninth abdominal segments (T3 through A9), or parasegments 5–14. These genes are expressed in overlapping portions of the embryo. *Ubx* is expressed in parasegments (PS) 5–13, *abdA* is expressed in PS7–13, and *AbdB* is expressed in PS10–14 (BEACHY *et al.* 1985; CELNIKER *et al.* 1989; KARCH *et al.* 1990).

Each BX-C gene has several regulatory regions which are responsible for proper gene expression in each parasegment (WHITE and WILCOX 1985; PEIFER *et al.* 1987; CELNIKER *et al.* 1990; KARCH *et al.* 1990). *Ubx* has two regulatory regions: the *abx/bx* region, named for mutations which affect PS5, and the *bx**d*/*pbx* region, where mutations affect PS6 (LEWIS 1978). The *abdA* regulatory mutations, *iab-2*, *iab-3* and *iab-4*, affect PS7, 8 and 9, and the *AbdB* regulatory mutations, *iab-5* through *iab-9*, affect PS10–14 (DUNCAN 1987; CELNIKER *et al.* 1990). The regulatory mutations also have subtle effects in more posterior parasegments. These regulatory regions are in order along the chromosome (see Figure 1). This unusual organization of the BX-C led to the proposal by PEIFER *et al.* (1987) that these regulatory regions function as isolated domains of chromosome structure, becoming activated in the appropriate parasegment and remaining open in more posterior parts of the fly.

The initial boundaries of homeotic gene expression are likely to be established by the action of gap and pair-rule genes (QIAN *et al.* 1991; MÜLLER and BIENZ 1992; SHIMELL *et al.* 1994). After these gene products decay in early embryogenesis, the roles of maintenance of homeotic gene repression and expression are taken over by the genes of the *Polycomb* and *trithorax* groups, respectively. The *Polycomb* group (Pc-G) of genes are required for maintaining repression of the homeotic genes. Mutations in these genes result in expression of the homeotic genes beyond their normal boundaries (WHITE and WILCOX 1985; STRUHL and AKAM 1985; CELNIKER *et al.* 1990; SIMON *et al.* 1992). Mutations in the *trithorax* group result in the loss of expression of homeotic genes in specific cells, tissues or parasegments (BREEN and HARTE 1993).

*P* elements containing the *lacZ* gene have been used as enhancer detectors in *Drosophila* (O'KANE and GEHRING 1987). For several of such "enhancer trap" insertions, their positions relative to the adjacent transcription unit have been mapped, and the pattern of the endogenous transcript has been compared to the pattern of  $\beta$ -galactosidase ( $\beta$ -gal) from the *P* element. For example, enhancer traps in the *elav*, *fasciclin III*, *Couch potato* and *connectin* genes have all been mapped to the 5' end of the endogenous transcript, and the *lacZ* patterns look very similar to the protein or RNA patterns produced by these genes in wild-type embryos (BIER *et al.* 1989; WILSON *et al.* 1989; BELLEN *et al.* 1992; NOSE *et al.* 1992). For other enhancer traps that have been characterized,  $\beta$ -gal is produced in a subset of the tissues

that express the adjacent gene (e.g., *Toll*, *rhomboid* (BELLEN *et al.* 1989; BIER *et al.* 1990)). Two enhancer traps have been characterized in the homeotic gene clusters. One is inserted just upstream of the *Antennapedia* P1 promoter and mimics the expression pattern of this transcript (ENGSTROM *et al.* 1992). Another has been mapped to the *iab-7* regulatory region of *AbdB*, and it appears to respond only to enhancers from within this regulatory region (GALLONI *et al.* 1993).

In this report, we describe eight *P* element enhancer traps that are inserted in the BX-C. Three of these lines behave like enhancer traps in most other loci and mimic the pattern of the adjacent gene. Five enhancer traps, however, do not reproduce the patterns of the homeotic genes, but they faithfully mark the parasegmental limits of the regulatory domains.

## MATERIALS AND METHODS

**Drosophila strains:** Insertions in the BX-C were identified by phenotype or  $\beta$ -gal pattern. Many carried DNA fragments from the complex (see Table 1) cloned into the *SaII* site of pMBO140 (SIMON *et al.* 1990). The transformant line  $bx^{Plac(-61)}$  (3-ry<sup>128</sup>) was kindly provided by CHRISTIAN KLÄMBT and COREY GOODMAN; the  $Ubx^{Plac(-31)}$  strain was kindly provided by JEFF SIMON. The *trx<sup>B11</sup>* mutation (MAZO *et al.* 1990) was recombined onto the  $bx^{Plac(-61)}$  and  $bxd^{Plac(+13)}$  chromosomes. The following Pc-G mutations were used: *esc<sup>2</sup>*, *esc<sup>10</sup>*, *Pc<sup>3</sup>* and *Pcl<sup>D5</sup>* (LINDSLEY and ZIMM 1992). *esc<sup>-</sup>* embryos were generated by crossing *esc<sup>2</sup>/esc<sup>10</sup>* females to *esc<sup>2</sup>/esc<sup>10</sup>* males that were heterozygous for one of the enhancer traps on the third chromosome. The  $\Delta 2-3$  chromosome (ROBERTSON *et al.* 1988) used in generating derivative lines contains a stably integrated source of *P* transposase.

**Mapping of *P* elements:** The *P* element insertions  $bx^{Plac(-61)}$ ,  $Ubx^{Plac(-61)}$ ,  $Ubx^{Plac(-31)}$  and  $bxd^{Plac(-31)}$  were recovered from bacteriophage  $\lambda$  genomic libraries. The libraries contained genomic DNA fragments generated by partial digestion with *Sau3AI*; these were inserted into the EMBL3 vector. The *P* element insertions  $bxd^{Plac(-31)}$ ,  $bxd^{Plac(+13)}$ ,  $iab-7^{Plac(+132)}$  and  $iab-8^{Plac(+159)}$  were mapped using inverse PCR (OCHMAN *et al.* 1988), using primers homologous to the 3' end of the *P* element. The resulting product was radiolabeled using PCR and was hybridized to a Southern blot containing *EcoRI*-digested phage clones carrying genomic DNA from the BX-C. The orientations of the *P* elements were determined by Southern blot. The junction fragments from several *P* element lines were sequenced using the Sequenase protocol (U.S. Biochemical Corp.).

**Antibody staining:** Embryos were fixed, stained and dissected as described (KARCH *et al.* 1990) using a mouse monoclonal against  $\beta$ -gal (Promega), followed by an HRP-conjugated goat-anti-mouse secondary antibody (Bio-Rad). The gut and visceral mesoderm are pulled away from the epidermis in these dissections, and are not included in the photographs here. X-gal staining of larval tissues was performed as in GLASER *et al.* (1986). The anti-UBX antibody was the FP.3.38 monoclonal (WHITE and WILCOX 1984), generously provided by IAN DUNCAN. The anti-ABDB was the 1A2E9 monoclonal (CELNIKER *et al.* 1989) generously provided by SUE CELNIKER.

**Detection of RNA *in situ*:** A 4.1-kb *EcoRI-HindIII* fragment from the *rosy* gene (coordinates 0.0 to +4.1, (COTÉ *et al.* 1986)) was cloned into pGEM (Promega). This plasmid was digested with *PstI*, which cuts 1.1 kb downstream from the

*EcoRI* site, and a digoxigenin-labeled RNA probe was produced using SP6 polymerase (New England Biolabs) following directions from the Genius kit (Boehringer Mannheim). The resulting probe is labeled from +1.1 to +4.1, which corresponds to a region that is deleted in the *ry<sup>506</sup>* strain (COTÉ *et al.* 1986). Antisense *lacZ* was generated using pGEM4lacZ, kindly provided by FRANCISCO PELIGRI. Subsequent treatment of the RNA probe and hybridization to embryos was performed using the procedure of GAVIS and LEHMAN (1992).

**Generation of derivative lines:** The  $Ubx^{Plac(-61)}$  insertion was derived from the  $bx^{Plac(-61)}$  insertion through dysgenesis. The  $bx^{Plac(-61)}$  was combined with the  $\Delta 2-3$  source of transposase for one generation in males, and the  $Ubx^{Plac(-61)}$  chromosome was recovered from a group of unselected male progeny.

Derivatives of  $iab-7^{Plac(+132)}$  were generated by crossing  $iab-7^{Plac(+132)}/Sb \Delta 2-3$  males to *ry<sup>-</sup>* females and recovering male *ry<sup>+</sup>* progeny that lacked patches of dark tissue on anterior abdominal segments. One derivative line ( $iab-7^{Plac(+132)\Delta}$ ) had the pattern shown in Figures 1G and 2, E and F. The structure of the *P* element inserted in the derivative line was determined by Southern blot using probes from the *iab-7*, *bxd/iab-2* and *Ubx* promoter regions.

$iab-8^{Plac(+159)}/Sb \Delta 2-3$  males were crossed to *ry<sup>502</sup>* females, and independent lines were established from *ry<sup>+</sup>* male progeny. Several derivative lines ( $iab-8^{Plac(+159)\Delta}$ ) had the *lacZ* pattern shown in Figures 1H and 3B.

## RESULTS

Figure 1 shows the embryonic *lacZ* expression patterns from eight enhancer trap lines, aligned according to their sites of insertion within the bithorax complex. The enhancer trap lines are named according to their phenotypes with the site of *P* element insertion (corresponding to the BX-C walk coordinates) in parentheses. Two of the *bxd* lines are inserted at approximately the same location (-31); these are indicated by A and B, referring to the order in which the lines were isolated. Deletion derivatives of initial *P* element lines are indicated with a  $\Delta$ . Three of the enhancer trap lines have insertions very close to the *Ubx* promoter (Figure 1, A, B and C). These lines have *lacZ* expression patterns that are very similar to that of the endogenous *Ubx* gene. The remaining five *P* elements, however, are inserted into the regulatory regions of *Ubx* (Figure 1, D, E and F) or *AbdB* (Figure 1, G and H). Two transformant lines express  $\beta$ -gal from the *P* element promoter, but the remaining lines express  $\beta$ -gal from the *Ubx* promoter (Table 1). The lines containing the *Ubx* promoter also contain various regulatory DNA fragments from the BX-C within the *P* elements (Table 1). These were recovered from a series of experiments where we were analyzing the regulatory DNA for enhancer properties (M. O'CONNOR and J. SIMON, unpublished results).

**The *Ubx* promoter insertions:** The enhancer traps shown in Figure 1, A, B and C, map just upstream of the *Ubx* promoter. The most proximal enhancer trap,  $Ubx^{Plac(-31)}$  (Figure 1A) is inserted 13 nucleotides upstream of the *Ubx* transcriptional start site (SAARI and BIENZ 1987). This insertion is homozygous lethal, and

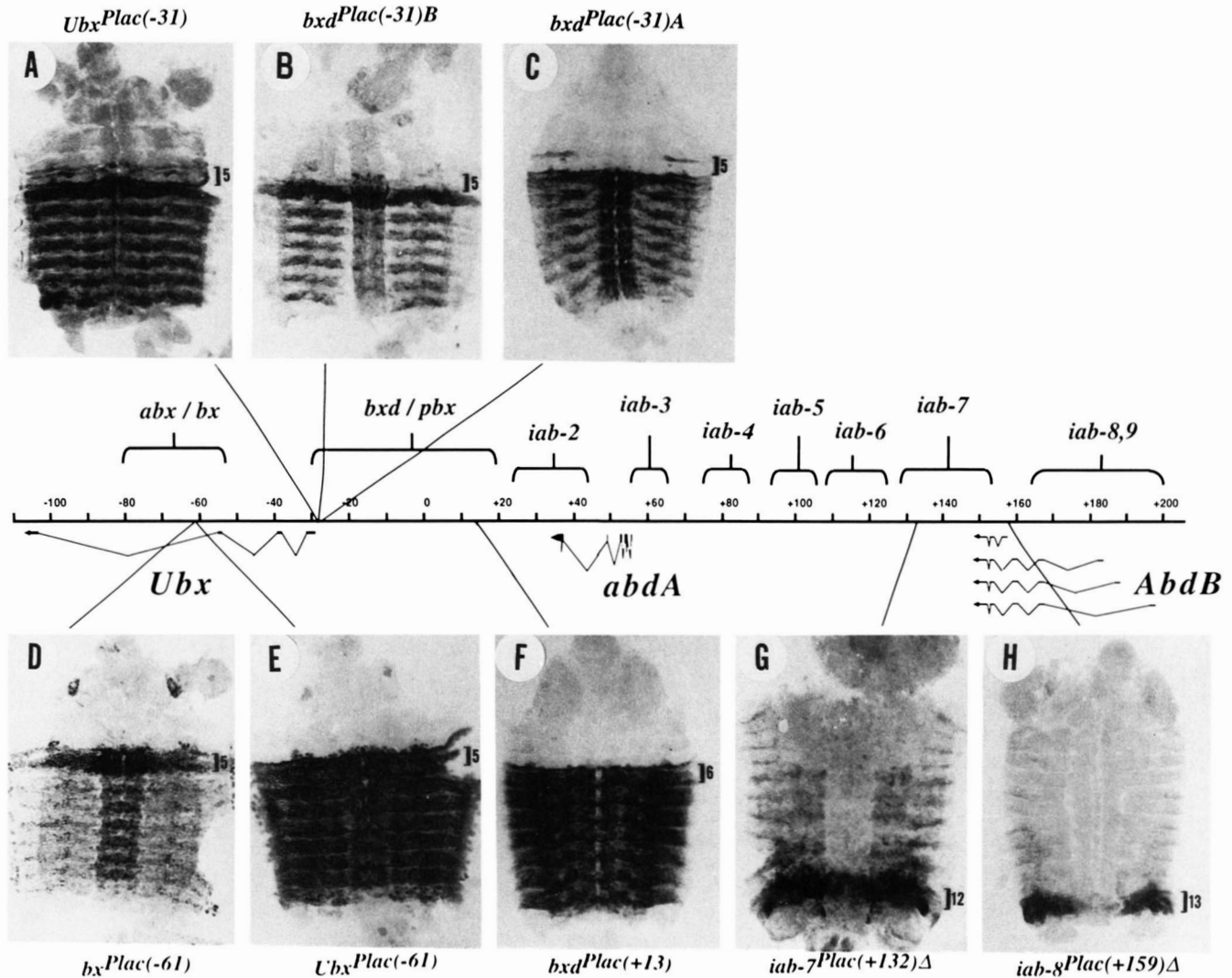


FIGURE 1.—Sites of insertion of the BX-C enhancer traps. Embryos were stained with an antibody against  $\beta$ -gal, were dissected and are oriented with anterior at the top. The number to the right of each embryo indicates the anteriormost parasegment where  $\beta$ -gal is expressed. The black lines point out the position of each enhancer trap relative to the BX-C map (coordinates are in kilobases). The *Ubx*, *abdA* and *AbdB* transcription units are shown below the line and the regulatory regions are shown above the line. Embryos are at the germ band retracted stage (10–12 hr). (A) *Ubx<sup>Plac(-31)</sup>*, (B) *bxd<sup>Plac(-31)B</sup>* and (C) *bxd<sup>Plac(-31)A</sup>* are P elements inserted at the *Ubx* promoter. (D) *bxd<sup>Plac(-61)</sup>*, (E) *Ubx<sup>Plac(-61)</sup>*, (F) *bxd<sup>Plac(+13)</sup>*, (G) *iab-7<sup>Plac(+132)Δ</sup>* and (H) *iab-8<sup>Plac(+159)Δ</sup>* are P elements inserted in the regulatory regions. The number in parentheses is the site of P element insertion relative to the BX-C map.

homozygous first instar larvae have a characteristic *Ubx<sup>-</sup>* phenotype, i.e., segment A1 is transformed to a thoracic segment and thoracic pits appear on the abdominal segments. The *Ubx* protein pattern resembles that of a strong *bxd* mutation (BEACHY *et al.* 1985; WHITE and WILCOX 1985) although it is also reduced in PS5 (data not shown).

The *bxd<sup>Plac(-31)B</sup>* insertion is 135 bp upstream of the *Ubx* start site (Figure 1B). Homozygotes die as pharate adults with a severe *bxd* transformation. Animals dissected from their pupal cases are missing the first abdominal tergite, have an additional band of postnotal tissue, and have halteres that are strongly transformed toward wing in the posterior compartment. Homozygous embryos have reduced levels of *Ubx* protein in PS6–12; the pattern in PS6 now resembles that of PS5 (data not shown).

The *bxd<sup>Plac(-31)A</sup>* P element (Figure 1C) is inserted 196 bp upstream of the *Ubx* start site. Heterozygotes over the *TM2* (*Ubx<sup>130</sup>*) balancer chromosome are viable, but the halteres are enlarged to about five times the size of *TM2/+*, and the first abdominal tergite is reduced in size. Homozygotes are similarly transformed but they have reduced viability and those that do survive usually have unexpanded wings. We have been unable to separate this wing phenotype from the *bxd* phenotype by recombination. Furthermore, some *ry<sup>-</sup>* derivatives of the *bxd<sup>Plac(-31)A</sup>* line, induced by hybrid dysgenesis, lack the wing phenotype. Both observations suggest that the wing phenotype is associated with the *bxd<sup>Plac(-31)A</sup>* insertion. The *Ubx* protein pattern in embryos appears normal.

TABLE 1  
P elements in the BX-C

Insertion	Isolate name	Promoter <sup>a</sup>	Regulatory DNA <sup>b</sup>
<i>bx</i> <sup>Plac(-61)</sup>	3-ry128	<i>P</i> (←)	None
<i>Ubx</i> <sup>Plac(-61)</sup>	3-ry128,42-4	<i>P</i> (→)	None
<i>Ubx</i> <sup>Plac(-31)</sup>	bx17J-36	<i>Ubx</i> (→)	17 kb <i>bx</i> -63 to -46
<i>bx</i> <sup>Plac(-31)A</sup>	UC31(1401)-116A	<i>Ubx</i> (→)	13 kb <i>bx</i> / <i>iab-2</i> +36 to +23
<i>bx</i> <sup>Plac(-31)B</sup>	UC42(1403)-96	<i>Ubx</i> (←)	18 kb <i>bx</i> / <i>iab-2</i> +30 to +12
<i>bx</i> <sup>Plac(+13)</sup>	UC47(1257)-121	<i>Ubx</i> (←)	7 kb <i>bx</i> / <i>iab-2</i> +23 to +30
<i>iab-7</i> <sup>Plac(+132)</sup>	UC43(1402)-7	<i>Ubx</i> (←)	13 kb <i>bx</i> / <i>iab-2</i> +30 to +22; +36 to +30
<i>iab-7</i> <sup>Plac(+132)Δ</sup>	UC43(1402-7),9III	<i>Ubx</i> Δ (←)	1 kb <i>bx</i> / <i>iab-2</i> +31 to +30
<i>iab-8</i> <sup>Plac(+159)</sup>	UC21-10	<i>Ubx</i> (→)	11 kb <i>iab-3</i> +67 to +56; 4 kb <i>Mcp</i> +99 to +95
<i>iab-8</i> <sup>Plac(+159)Δ</sup>	UC21-10,1-d	<i>Ubx</i> (→)	11 kb <i>iab-3</i> +67 to +56; 4 kb <i>Mcp</i> +99 to +95

<sup>a</sup> Direction of transcription of *lacZ* gene relative to the endogenous *Ubx* gene (←).

<sup>b</sup> *SalI* DNA fragments from the BX-C. Coordinates are from the BX-C walk; the first coordinate is adjacent to the *P* element end and the last coordinate is adjacent to the *Ubx* promoter in the pMBO140 construct.

All three of these enhancer traps have a  $\beta$ -gal expression pattern that mimics the *Ubx* protein pattern in several respects. They have expression in the epidermis and CNS from PS5-13, with the highest level of expression in PS6. All three lines have expression in the visceral mesoderm in PS7, and the two most proximal lines have expression in the CNS midline cells of PS4. The most distal insertion, *bx*<sup>Plac(-31)A</sup>, has much less staining in PS5 than the other two lines (Figure 1C).

In the three insertion lines (especially the *bx*<sup>Plac(-31)B</sup> line), young embryos express  $\beta$ -gal weakly in the lateral epidermis and head in a pattern similar to the basal pattern produced from the *Ubx-lacZ* construct (BIENZ *et al.* 1988; SIMON *et al.* 1990). But by 10 hr of development, there is no evidence of the basal pattern in these or any of the lines shown in Figure 1. We stained for *lacZ* RNA in embryos from three enhancer trap lines (*bx*<sup>Plac(-31)B</sup>, *bx*<sup>Plac(+13)</sup>, and *iab-8*<sup>Plac(+159)Δ</sup>) to measure more accurately the time of *lacZ* shut off. RNA expression in the head is last observed at about 6 hr of development (data not shown).

The *bx*<sup>Plac(-31)A</sup> and *bx*<sup>Plac(-31)B</sup> elements contain DNA fragments from the BX-C regulatory regions upstream of the *Ubx* promoter (Table 1). Control lines containing these constructs inserted outside the BX-C have  $\beta$ -gal patterns that initiate with a PS6 boundary in early embryos, but at late times  $\beta$ -gal is expressed throughout the embryo (data not shown). The position in the BX-C prevents the anterior expression. This position effect is also observed in the *bx*<sup>Plac(+13)</sup> and *iab-7*<sup>Plac(+132)</sup> lines and is discussed in more detail below. Similarly, *Ubx*<sup>Plac(-31)</sup> contains DNA from the *bx* region. At late stages of embryogenesis, insertions outside the BX-C that contain this *bx* fragment express  $\beta$ -gal in the anterior thorax and head, but this anterior expression does not occur in *Ubx*<sup>Plac(-31)</sup> embryos.

**The PS5 insertions:** The *bx*<sup>Plac(-61)</sup> *P* element is the PZ construct (MLODZIK and HIROMI 1992), with a *P* element promoter driving  $\beta$ -gal. The resulting enzyme includes the nuclear localization signal from the *P* element. The insertion maps at -61 kb on the BX-C map, within the

large *Ubx* intron. It is oriented with the *lacZ* gene transcribed in the same direction as the *Ubx* gene. Animals homozygous for the insertion have a very mild *bx* phenotype, with slightly enlarged halteres. Heterozygotes over a strong *Ubx* allele (such as *Ubx*<sup>130</sup> on the TM2 chromosome) show a slightly stronger transformation, with a few small wing bristles on the capitellum. The pattern of *Ubx* protein in homozygous embryos appears normal. There is strong and uniform *lacZ* staining in nuclei of PS5 and somewhat weaker cell-specific expression in PS6-12 (Figure 1D). There is also strong expression in the nuclei of the amnioserosa, and in a group of cells in the mandibular lobe of the head. There is no apparent staining of the visceral mesoderm.

The *Ubx*<sup>Plac(-61)</sup> insertion was derived from the *bx*<sup>Plac(-61)</sup> line by hybrid dysgenesis (see MATERIALS AND METHODS). This element is an exact inversion of the *bx*<sup>Plac(-61)</sup> element. Both junctions between the *P* element and the BX-C were sequenced, and the target site duplications and flanking sequences were identical in the two insertions. Although only the orientation of the element has been changed, the phenotype and *lacZ* patterns are dramatically altered. The *Ubx*<sup>Plac(-61)</sup> insertion is a strong *Ubx* mutation; homozygotes die as larvae, with a strong transformation of the first abdominal segment to a third thoracic type. Furthermore, homozygous embryos lack *Ubx* protein as detected with *Ubx* antibody. This *Ubx*<sup>-</sup> phenotype may be caused by premature termination of the *Ubx* transcript when the *P* element is in this orientation. The phenotype of *Ubx*<sup>Plac(-61)</sup> is probably not due to any second site mutations, since most *ry*<sup>-</sup> revertants induced by hybrid dysgenesis are wild type. The *lacZ* expression is strong and uniform in PS5-11, and somewhat weaker in PS12 (Figure 1E). In older embryos, the PS5 expression is slightly stronger than that of PS6-11, but there is not the clear difference between parasegments 5 and 6 seen in *bx*<sup>Plac(-61)</sup>, and the epidermal staining in PS6-11 appears to be virtually universal. As in the *bx*<sup>Plac(-61)</sup> line, there is also expression in the amnioserosa and the mandibular lobes, but not in the visceral mesoderm.

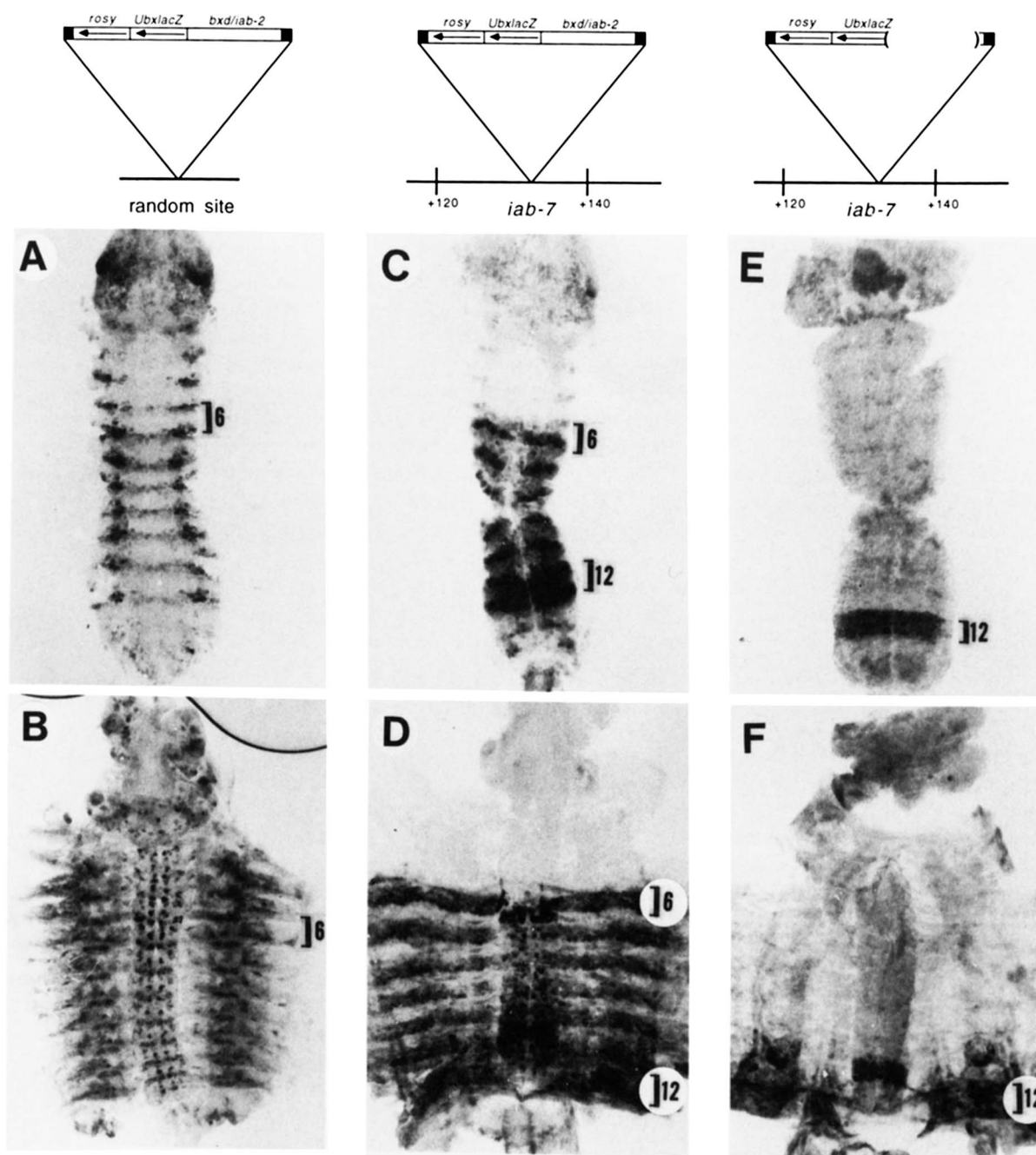


FIGURE 2.—Expression of  $\beta$ -gal from the *bxg/iab-2* construct in the *iab-7* region. The structure of the *P* elements and their sites of insertion are indicated above the embryos. Embryos in A, C and E are at the germ band extended stage (6 hr). Embryos in B, D and F are germ band retracted (15 hr). (A, B) Transformant line 1402-2, (C, D) *iab-7<sup>lac(+132)</sup>*, (E, F) *iab-7<sup>lac(+132)Δ</sup>*. Parasegments 6 and 12 are labeled.

**The PS6 insertion:** The *bxg<sup>lac(+13)</sup>* insertion maps in the *bxg* region at +13 on the BX-C map (Figure 1F). Homozygotes are fully viable, and they show only a slight reduction of the A1 tergite. The A1 reduction is enhanced in animals that are heterozygous for this *P* element and the *TM2* (*Ubx<sup>130</sup>*) balancer. The pattern of *Ubx* protein in homozygous embryos appears normal. The *lacZ* gene from this insertion is expressed strongly and uniformly in the epidermis and CNS of PS6–12 and these boundaries of expression are maintained through-

out embryogenesis. This expression pattern is appropriate for the *bxg* regulatory domain since *bxg* mutations affect PS6 and posterior parasegments. This line shows staining in the visceral mesoderm of PS7, and at later embryonic stages staining becomes stronger in the CNS of PS6.

**The PS12 insertion:** Our initial insertion in the *iab-7* region, called *iab-7<sup>lac(+132)</sup>*, is located at +132. The line programs a *lacZ* pattern with cell-specific expression in PS6–13, with enhanced staining in PS12 (Figure 2, C

and D). The *P* element contains DNA from the *bxd/iab-2* region (Table 1) which programs the anterior boundary in PS6. In control lines, where the construct is not in the BX-C, this boundary is transient, and there is substantial staining anterior to PS6 at late embryonic stages (Figure 2, A and B). In the *iab-7* line, however, the PS6 boundary is much sharper, and is maintained throughout embryogenesis (Figure 2, C and D). Early embryos show some anterior expression in the basal *Ubx-lacZ* pattern (Figure 2C), but the basal pattern disappears by the time of germ band retraction, as in the *Ubx* promoter enhancer traps. Thus, the position in the *iab-7* region prevents expression of  $\beta$ -gal anterior to PS6.

Adult males that are heterozygous for this insertion have patches of dark pigment on anterior abdominal tergites, similar to the *Mcp* mutation (LEWIS 1978). Homozygous males have more and larger patches of ectopic pigmented tissue, have a sixth tergite that is reduced in size (like *Fab7*) (GYURKOVICS *et al.* 1990), and have a partial seventh tergite (like weak *iab-7* mutations). Thus, these flies have a gain-of-function (like *Mcp*, *Fab7*) and a loss-of-function (*iab-7*) phenotype. The gain-of-function phenotypes may result from ectopic activation of the *AbdB* gene by the *bxd/iab-2* sequences. No ectopic *AbdB* expression was observed in embryos, but there could be misexpression later in development.

We used hybrid dysgenesis to delete the internal *bxd/iab-2* sequences, selecting for *ry*<sup>+</sup> derivative lines that were reverted for the dominant phenotype. One derivative line (*iab-7*<sup>Plac(+132) $\Delta$</sup> , Figures 1G and 2, E and F) has a deletion of most of the *bxd/iab-2* DNA (see map in Figure 2). Homozygotes of this line have only the *iab-7* loss-of-function phenotype; males have a small seventh tergite. The *bxd/iab-2* pattern is absent in this line, and *lacZ* is expressed in the epidermis, mesoderm and CNS of PS12 (Figures 1G and 2, E and F). This is the expected expression domain, since *iab-7* mutations primarily affect PS12. The PS12 stripe of the *iab-7* insertion is maintained throughout embryogenesis and is especially sharp at late stages in the CNS (Figure 2F). There is anterior epidermal expression observed in some embryos (Figure 1G) that may be due to the remaining *bxd/iab-2* DNA on the *P* element, but this anterior expression is absent at 12 hr.  $\beta$ -gal is also expressed in the visceral mesoderm, the tracheal tubes and the posterior gonad (Figure 2F). This pattern is very similar to the *btl* insertion at +124.7, reported by GALLONI *et al.* (1993). The principal difference is that the *btl* insertion expresses  $\beta$ -gal in PS12–14, whereas *iab-7*<sup>Plac(+132) $\Delta$</sup>  does not have significant  $\beta$ -gal expression posterior to PS12. We have examined an additional insertion of the "PZ" element in the *iab-7* region, discovered by LYNNE SCHNEIDER and ALLAN SPRADLING. Its *lacZ* expression pattern resembles that of *iab-7*<sup>Plac(+132) $\Delta$</sup>  or *btl* (data not shown).

**The PS13 insertion:** The initial insertion in the *iab-8*

region (*iab-8*<sup>Plac(+159)</sup>, Figure 3A) was mapped to +159 on the BX-C map. Two distinct bands were generated by inverse PCR from the *P* element 3' end, and each was cloned and sequenced. One contained the 3' *P* element sequence and adjacent *AbdB* sequence, placing the *P* element 253 bp upstream of the proximal *AbdB* promoter (*AbdB-m* or *AbdB-I*) (ZAVORTINK and SAKONJU 1989; CELNIKER *et al.* 1990). The other cloned fragment contained the 3' *P* element sequence adjacent to plasmid vector sequence. Southern blot analysis confirmed that pUC8 sequences were integrated along with two *P* element constructs at *AbdB* (map in Figure 3A). This double insertion is homozygous lethal. The homozygous first instar larvae lack posterior spiracles, and the A8 denticle belt is transformed toward a more anterior denticle belt. Some larvae are more severely transformed, with a partial A9 denticle belt and chitinous plates near the anal pads. *AbdB* protein appears reduced in all parasegments of homozygous embryos, especially PS10–13 (data not shown).

The *iab-8* insertion contains DNA from the *Mcp* and *iab-3* regions (Table 1, map in Figure 3). Control lines with this construct outside the BX-C show the basal *Ubx-lacZ* pattern, with occasional embryos showing a weak PS8 boundary, which is normally programmed by this *iab-3* DNA fragment [as in the *iab-3-11.5* construct of SIMON *et al.* (1990)]. The double insertion at the *iab-8* site (*iab-8*<sup>Plac(+159)</sup>), however, expresses the PS8 pattern strongly, as well as aspects of the *Ubx-lacZ* basal pattern (Figure 3A). In addition, there is a strong stripe of expression in PS13.

We generated several derivatives of this line that contain only one copy of the *P* element (see MATERIALS AND METHODS). These lines are still homozygous lethal. The phenotype resembles that of the double insertion, except none of the first instar cuticles have a partial A9 denticle belt or chitinous plates. *AbdB* protein appears to be expressed at normal levels in PS14 but is reduced in PS10–13 of homozygous embryos (data not shown). The  $\beta$ -gal pattern from a single *P* element line, *iab-8*<sup>Plac(+159) $\Delta$</sup> , is shown in Figures 1H and 3B. This line stains intensely in the epidermis and CNS of PS13, and most of the anterior staining seen in the double insertion is absent. It is striking that this insertion is so close to the *AbdB* promoter, yet the pattern does not look like the *AbdB* expression pattern. The transcript from the *AbdB-I* promoter is expressed in PS10–13 in wild-type embryos (KUZIORA and MCGINNIS 1988; SANCHEZ-HERRERO and CROSBY 1988). Some embryos have weak  $\beta$ -gal expression in the epidermis of PS8–12 (Figure 3B) which may be due to the *iab-3* fragment on the *P* element or weak response to *iab-5*, -6 and -7 enhancers, but, in any case, there is no staining anterior to PS13 in the CNS.

**Expression in Pc-G and *trx* mutants:** Mutations in the *trithorax* locus cause a reduction in the pattern of *Ubx*

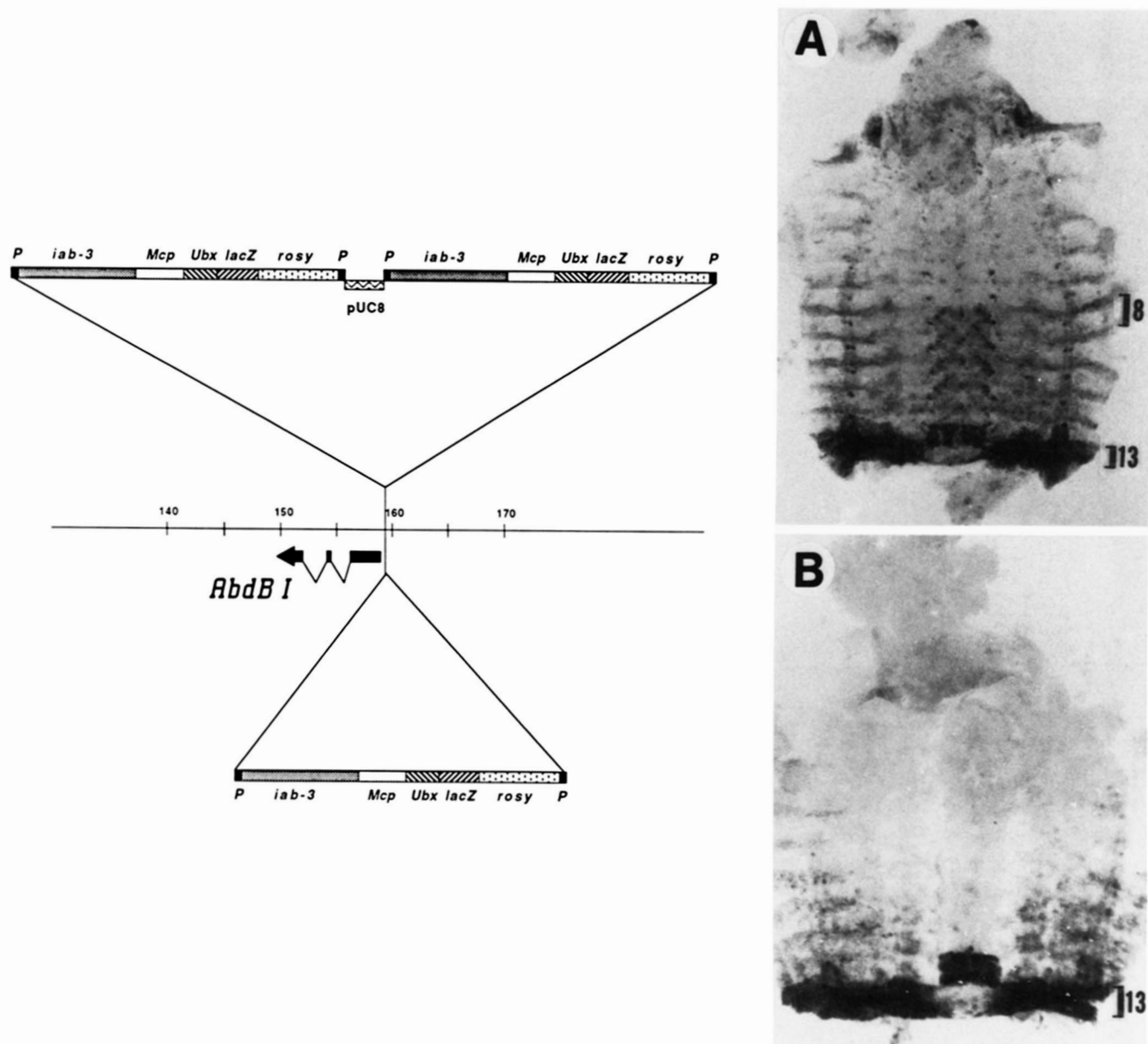


FIGURE 3.—Comparison of single and double *P* element insertions in the *iab-8* region. (A)  $\beta$ -gal pattern of the double insertion line *iab-8<sup>Plac(+159)</sup>*. The structure of the double *P* element is diagrammed to the left of the embryo. (B)  $\beta$ -gal pattern from the single insertion line *iab-8<sup>Plac(+159)Δ</sup>*. The structure of the *P* element is diagrammed below the line. Parasegments 8 and 13 are indicated.

expression (MAZO *et al.* 1990; BREEN and HARTE 1993). We have looked at two enhancer traps from the *Ubx* regulatory regions for changes in their expression patterns in *trx<sup>B11</sup>* mutant embryos (Figure 4, B and E). Both lines show cell-specific reductions in  $\beta$ -gal expression. The most dramatic reduction is observed in PS5 of *bx<sup>Plac(-61)</sup>*, where  $\beta$ -gal is expressed at high levels in *trx<sup>+</sup>* embryos but at a much lower level in *trx<sup>B11</sup>* embryos (compare Figure 4, A and B). *trx<sup>B11</sup> bxd<sup>Plac(+13)</sup>* embryos (Figure 4E) have a less uniform  $\beta$ -gal expression pattern than *trx<sup>+</sup>* embryos (Figure 4D), with the highest  $\beta$ -gal levels at the segment borders.

Mutations in the Polycomb group (Pc-G) cause the homeotic genes to spread beyond their initial segmental

boundaries. Figure 4, C and F, shows the expression pattern of two transformant lines in embryos that are mutant for the *extra sex combs* gene (*esc*, a Pc-G member). The embryos shown lack both maternal and zygotic *esc* function (STRUHL and AKAM 1985). The *lacZ* expression in these enhancer trap lines appears in the normal position in early embryos, but it spreads beyond the normal anterior and posterior boundaries during the extended germ band stage (~5 hr). The *Ubx*, *abdA* and *AbdB* genes spread beyond their normal domains in *esc<sup>-</sup>* embryos at about the same time in development (STRUHL and AKAM 1985; SIMON *et al.* 1992). The other insertions shown in Figure 1 have also been examined in *esc* null backgrounds, and all show *lacZ* pattern expansion

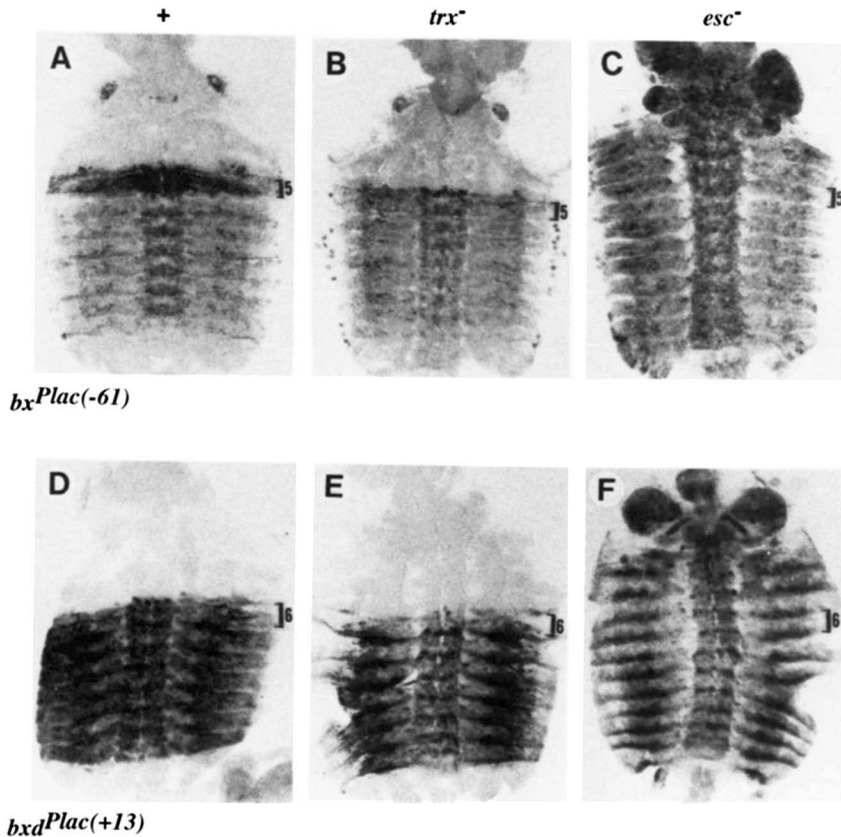


FIGURE 4.— $\beta$ -gal expression of BX-C enhancer traps in *trx* and *esc* mutant backgrounds. All embryos are at the germ band retracted stage at about twelve hr of development. (A) *bxd<sup>Plac(-61)</sup>* homozygote, (B) *trx<sup>B11</sup> bxd<sup>Plac(-61)</sup>* homozygote, (C) embryo from *esc<sup>10</sup>/esc<sup>2</sup>; bxd<sup>Plac(-61)</sup>/+* parents, (D) *trx<sup>B11</sup> bxd<sup>Plac(+13)</sup>/+* heterozygote, (E) *trx<sup>B11</sup> bxd<sup>Plac(+13)</sup>/trx<sup>B11</sup>* embryo, (F) embryo from *esc<sup>10</sup>/esc<sup>2</sup>; bxd<sup>Plac(+13)</sup>/+* parents. Parasegments 5 and 6 are indicated.

throughout the body axis. Four insertions (*bxd<sup>Plac(-61)</sup>*, *bxd<sup>Plac(+13)</sup>*, *iab-7<sup>Plac(+132)</sup> $\Delta$*  and *iab-8<sup>Plac(+159)</sup> $\Delta$* ) have been tested in embryos lacking zygotic *Polycomb-like*, and one (*bxd<sup>Plac(-31)</sup>A*) has been examined in embryos lacking zygotic *Polycomb*; all five cases show *lacZ* anterior to the normal boundaries.

**Expression from the *rosy* promoter:** All of the *P* elements in this study carry the *rosy* gene as a marker for transformation. We examined *rosy* expression in three enhancer traps. Two of these (*bxd<sup>Plac(+13)</sup>* and *iab-7<sup>Plac(+132)</sup> $\Delta$* ) carry a deletion at the *rosy* locus (*ry<sup>506</sup>*). Thus, all *rosy* expression observed in these lines comes from the copy of *rosy* carried on the *P* elements. Wild-type embryos express *rosy* in a broad ventral domain at the blastoderm stage and in the anterior and posterior midgut invaginations during gastrulation and germ band extension (DOYLE *et al.* 1989; data not shown). After germ band retraction, some embryos express *rosy* in the gut underlying PS8 and there is very weak staining in the malpighian tubules (data not shown). There is no detectable *rosy* mRNA in the epidermis, somatic mesoderm or CNS of wild type embryos after germ band retraction. The three enhancer trap lines express *rosy* in the wild-type pattern combined with new domains of expression beginning at the blastoderm stage. In the *bxd<sup>Plac(+13)</sup>* line, *rosy* mRNA is expressed in a new domain with a PS6 boundary (Figure 5A), although the pattern is not as uniform as the  $\beta$ -gal pattern (Figure 1F). *iab-7<sup>Plac(+132)</sup> $\Delta$*  expresses *rosy* in PS12 and more posterior

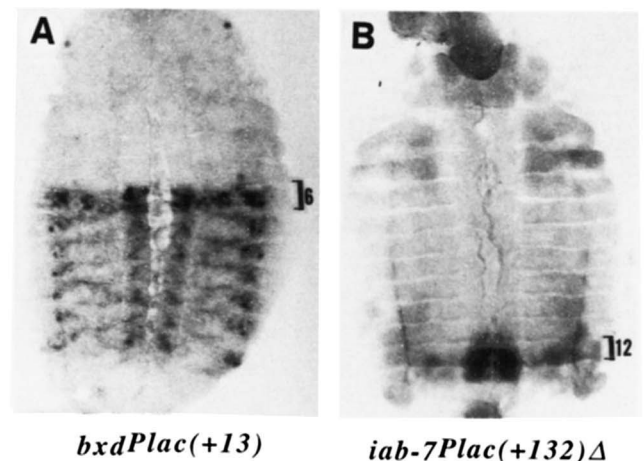


FIGURE 5.—Expression from the *rosy* gene in BX-C enhancer traps. *rosy* mRNA was detected using a digoxigenin-labeled antisense-*rosy* RNA probe. (A) *bxd<sup>Plac(+13)</sup>*, (B) *iab-7<sup>Plac(+132)</sup> $\Delta$* . Parasegments 6 and 12 are indicated.

parasegments (Figure 5B); the  $\beta$ -gal pattern (Figure 1G) is limited to PS12. In this line, *rosy* is expressed at much higher levels in the CNS than in other tissues. *bxd<sup>Plac(-61)</sup>* embryos express *rosy* in PS5–12 at early times, but by germ band retraction *rosy* appears only in lateral spots in PS5 and PS6 (data not shown). Although the cell and tissue specificity differs between *rosy* and *Ubx-lacZ* in these enhancer traps, the anterior boundaries of expression are the same.

**$\beta$ -gal expression in the larva:** We examined the  $\beta$ -gal patterns in the third instar larval CNS to determine if the

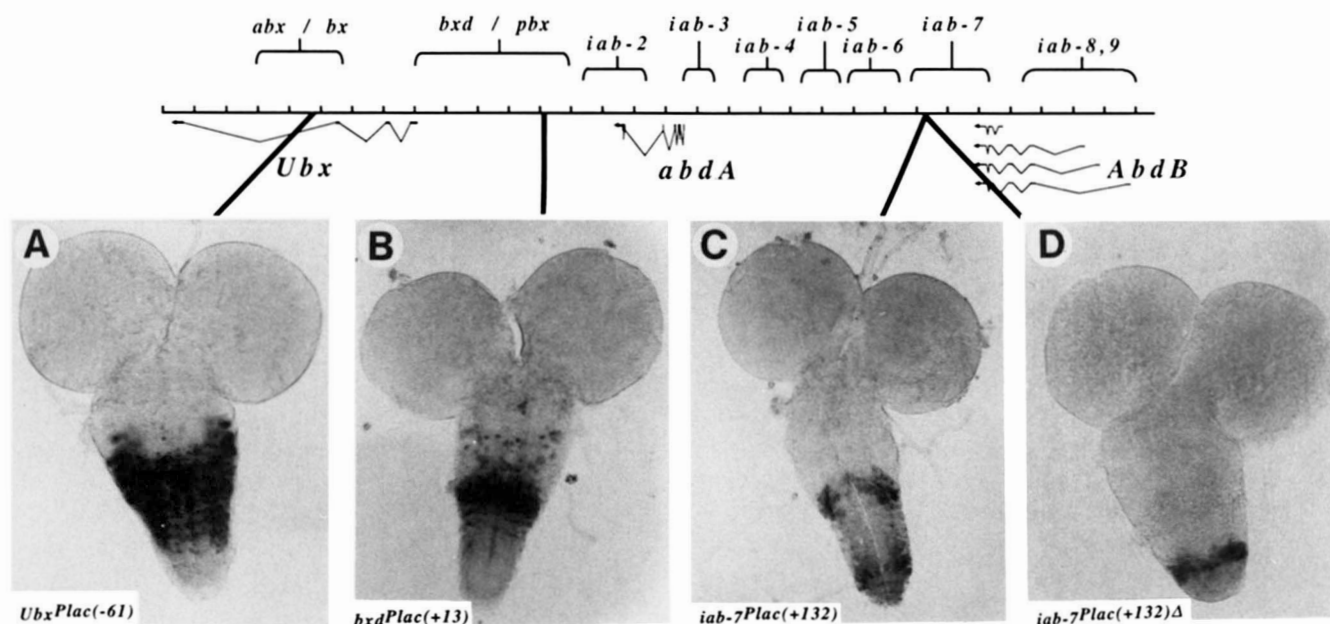


FIGURE 6.—Expression of  $\beta$ -gal in the larval CNS. Wandering third instar larvae were dissected and stained with X-gal. (A)  $Ubx^{Plac(-61)}$ , (B)  $bxd^{Plac(+13)}$ , (C)  $iab-7^{Plac(+132)}$ , (D)  $iab-7^{Plac(+132)\Delta}$ .

pattern boundaries are maintained. All the BX-C enhancer traps shown in Figures 1 and 2 were stained; four are shown in Figure 6. Parasegmental boundaries were confirmed by double staining with monoclonal antibody BP101, which labels the segmental ganglia (data not shown). The CNS from a  $bxd^{Plac(+13)}$  larva stains in a few cells ahead of the obvious stripe in PS6 (Figure 6B). With the exception of these few cells, the larval CNS patterns in all lines resembled the late embryonic CNS patterns, with strict maintenance of the anterior boundaries.

#### DISCUSSION

**Traps sense local enhancers?:** A simple view of enhancer traps is that they respond to regulation from nearby enhancers. The mechanisms of enhancer function are only partly understood. In some cases, transcription factors (such as the GAL4 or GCN4 proteins in *Saccharomyces cerevisiae*) bind to a site near the promoter and interact with basal transcription machinery. These factor binding sites must be within a few kb of a promoter to exert their effect (SADOWSKI *et al.* 1988). Some promoters clearly interact with regulatory sequences over much larger distances. Perhaps distant enhancers are brought close by looping (DUNN *et al.* 1984; BENSON and PIRROTTA 1988). If the long-distance regulation requires special DNA sites at or near the promoter, then an enhancer trap might respond to distant regulatory sequences only if the mobile element inserts near a natural promoter. It is common for *P* elements in *Drosophila* to insert very close to endogenous transcription start sites, and such promoter-proximal *P* enhancer traps often reproduce the expression pattern of the endogenous gene. The three insertions which lie immediately upstream of the *Ubx* transcription start site are typical of

this group; they give patterns similar to the normal pattern of *Ubx* expression. But the other five insertions do not reproduce the patterns of the endogenous genes, and, with the exception of  $iab-8^{Plac(+159)\Delta}$ , they do not lie near known endogenous promoters. It is difficult to explain their patterns as a response to transcription factors bound near the insertion sites, for several reasons.

**Uniform *lacZ* expression:** Each enhancer trap in the regulatory regions expresses *lacZ* in the appropriate parasegment, and the expression is fairly uniform in every case. For the enhancer traps marking PS5, the *lacZ* patterns are clearly different from the pattern of the endogenous *Ubx* gene, which is expressed only in a small subset of PS5 cells. Moreover, the regulatory fragments of the bithorax complex that have been tested typically do not drive uniform expression within a parasegment. The “*abx*” enhancer, for example, gives a graded pattern across the epidermis of PS5, and marks a subset of cells in the central nervous system (SIMON *et al.* 1990; MÜLLER and BIENZ 1991). We have examined the *lacZ* RNA pattern in the  $Ubx^{Plac(-61)}$  line to determine if the uniform expression is due to the perdurance of  $\beta$ -gal protein from early stages. The *lacZ* RNA in PS5 remains widespread in the epidermis and virtually universal in the cells of the CNS throughout embryonic development (data not shown). The  $\beta$ -gal expression pattern in the larval CNS suggests that the uniform expression continues through later stages of development (Figure 6A).

**Three different promoters:** The regulatory elements that turn on the enhancer traps in specific parasegments appear not to require a special promoter for their action. In six examples discussed here, the *Ubx* promoter is directing the expression of  $\beta$ -gal. This is the appropriate promoter for insertions near the natural *Ubx* RNA

start site, and for the insertion at +13 in the *bx*d region. But the *iab-7* and *iab-8* insertions are responding to information that normally directs an *AbdB* promoter. The two insertions at -61, in the *bx* region, have *P* element promoters driving  $\beta$ -gal. Moreover, the promoter for the *rosy* transcription unit, in the three cases examined (Figure 5), is also turned on in the appropriate parasegments.

**Action at a distance:** Although we have not mapped the regulatory sequences that drive any of the *lacZ* patterns described here, it is clear that they must act at a considerable distance. The six elements which contain the *Ubx* promoter fused to *lacZ* have various DNA fragments inserted into the *P* element upstream of the promoter. There is between 3.4 and 20 kb of DNA between the *Ubx/lacZ* RNA start site and the upstream edge of the *P* element. The downstream edge of the *P* element is 12 kb from the *Ubx/lacZ* RNA start site. In the examples of *rosy* promoter regulation, the *rosy* RNA start site (CURTIS 1990) lies 15.4 or 9.4 kb (Figure 5, A and B, respectively) from the upstream edge of the *P* element, and 6.0 kb from the downstream edge.

**Patterns do not reflect flanking enhancers:** The DNA flanking these enhancer traps has been tested for enhancer properties using a *lacZ* reporter system (SIMON *et al.* 1990; QIAN *et al.* 1991; MÜLLER and BIENZ 1991; BUSTURIA and BIENZ 1993; M. O'CONNOR, unpublished results) and the patterns generated from these constructs do not look like the patterns from our enhancer traps. For example, an element only 2 kb to the left of the *P* element promoter in the *Ubx<sup>Plac(-61)</sup>* insertion has been analyzed in detail, and has been shown to drive  $\beta$ -gal in a PS6 restricted pattern (QIAN *et al.* 1991), in contrast to the PS5 boundary of the enhancer traps. The closest PS5 element that has been mapped (the "*abx*" element) is 17 kb to the left of the PS5 enhancer traps (SIMON *et al.* 1990; MÜLLER and BIENZ 1991). Thus, the enhancer traps must be responding to parasegmental regulation specified at sites distant from the edge of the *P* element, but the traps do not recognize nearby or distant enhancers that specify cell types.

**Suppression of internal enhancers:** The "position effects" of the bithorax complex not only turn on new patterns from the enhancer trap *P* elements, they also turn off patterns that the *P* elements would normally express. The *Ubx* promoter/*lacZ* fusion present on most of our enhancer traps (Table 1) has been shown to produce a "basal" pattern of weak *lacZ* expression in the lateral epidermis and head when it is inserted at random in the chromosomes (BIENZ *et al.* 1988; SIMON *et al.* 1990). We have noted this basal pattern in insertions outside the bithorax complex of the *P* elements of the *Ubx<sup>Plac(-31)</sup>*, *bx<sup>d</sup><sup>Plac(+13)</sup>*, *iab-7<sup>Plac(+132)</sup>*, and *iab-8<sup>Plac(+159)</sup>*Δ enhancer traps. The same basal pattern is frequently seen in random insertions of the PZ element, with the *P* promoter driving *lacZ* (MLODZIK and HIROMI 1992).

The basal *lacZ* pattern is repressed in all eight lines shown in Figure 1. This is seen most clearly for the *iab-7<sup>Plac(+132)</sup>* element when inserted inside and outside the bithorax complex (Figure 2, A and C). There is a prominent spot of expression in the mandibular lobe of the head in the *bx<sup>Plac(-61)</sup>* line and much weaker expression in the same place in the *Ubx<sup>Plac(-61)</sup>* line. This spot persists through embryonic development, and represents the only apparent exception to the repression of *lacZ* in the anterior segments.

The normal level of *rosy* expression in embryos is quite low, and it is not clear whether the *rosy* gene internal to the enhancer traps is turned off in the more anterior parasegments. However, all enhancer traps recovered within the BX-C carry *rosy<sup>+</sup>* as a transformation marker; none carry *white<sup>+</sup>*, although several large screens have been performed using *white<sup>+</sup>* *P* elements (BIER *et al.* 1989; NOSE *et al.* 1992). *rosy* is a non-autonomous gene (HADORN and SCHWINCK 1956), but *white* is a cell-autonomous gene in the eye disc, an anteriorly derived structure (BEADLE and EPHRUSSI 1936). The *white* gene inserted into the BX-C might be repressed, and such a transformant would be undetectable.

**Patterns are maintained by Polycomb group, not by feedback from homeotics:** The parasegmental boundaries of the homeotic genes appear to be set by the gap and pair-rule regulators (QIAN *et al.* 1991; SHIMELL *et al.* 1994). The *lacZ* patterns of the enhancer traps are likely specified by the gap and pair-rule genes, not by the homeotics. The enhancer traps express  $\beta$ -gal before *Ubx* or *AbdB* proteins are detectable (data not shown), and their cell-specificity differs from that of *Ubx* or *AbdB*. The *Ubx<sup>Plac(-61)</sup>* insertion is an apparent *Ubx* null mutation, and the *iab-8<sup>Plac(+159)</sup>* insertion causes a strong *AbdB* mutation, but in both cases, embryos homozygous for the insertion (*i.e.*, lacking *Ubx* or *AbdB*) give *lacZ* patterns identical to heterozygotes (although more intense). We have also shown that the *bx<sup>Plac(-61)</sup>* pattern is unchanged in a *Ubx<sup>-</sup>* background (data not shown).

If the enhancer traps are responding to the gap and pair-rule signals, then some mechanism must maintain the pattern boundaries after the gap and pair-rule stripes disappear, at 3–4 hr of embryogenesis. That mechanism involves the genes of the *Polycomb* family, since the anterior restriction of  $\beta$ -gal is lost in Pc-G mutants (Figure 4, C and F). The high level of expression of  $\beta$ -gal in PS5 in *bx<sup>Plac(-61)</sup>* embryos is dependent on the *trithorax* gene (Figure 4B), which may play an antagonistic role to the *Polycomb* family (INGHAM 1983).

**Chromosome structure alternative:** It has been suggested that the bithorax complex includes a series of domains whose chromosome structure differs from segment to segment (PEIFER *et al.* 1987). This accessibility model helps to explain the sequential order of regulatory regions, and the phenotypes of some mutations

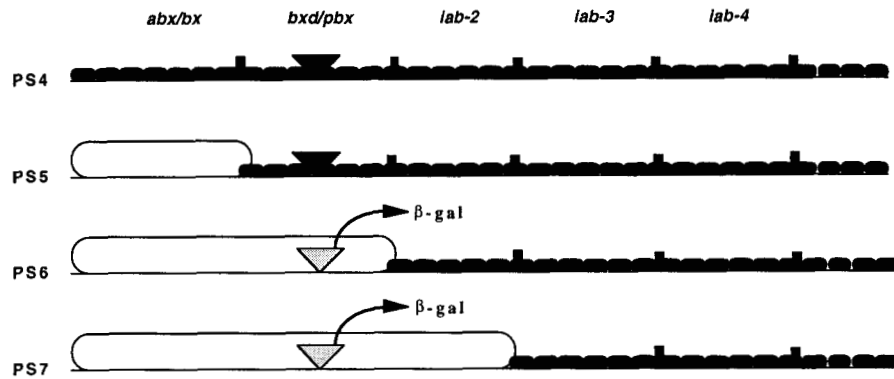


FIGURE 7.—Chromosome accessibility model. Each line depicts the bithorax complex in one parasegment for parasegments 4 through 7. The triangle represents the  $bxd^{Plac(+13)}$   $P$  element. The black ovals represent the *Polycomb* group of gene products binding to the BX-C, making the DNA inaccessible to transcription factors. In PS5, the *bx* region is active (shown by the open oval) and the *Ubx* gene is transcribed, but the *bxd* region is still repressed by the Pc-G. In PS6 the *bxd* region is accessible to transcription factors and the *lacZ* gene is transcribed (indicated by the arrow producing  $\beta$ -gal). More distal regions of the BX-C become progressively accessible in more posterior parasegments.

caused by rearrangements. Figure 7 diagrams consequences of the model for one of the enhancer traps, the  $bxd^{Plac(+13)}$  trap in the PS6 regulatory domain. In the head and parasegments 1–5, this region of the complex would have proteins of the Polycomb family bound to it, and it might be sterically unable to interact with RNA polymerase and transcription factors (PARO 1990; EISENBERG and ELGIN 1991). This view is supported by the protein similarity between Polycomb and the HP1 heterochromatin protein (PARO 1990), as well as by the demonstrated binding of Polycomb protein to the bithorax complex in a segment where the complex is repressed (ZINK and PARO 1989). In parasegment 6 (as well as PS7–13), the structure of the chromosome around the +13 enhancer trap is altered so that transcription factors have easy access. In the most simple and extreme view, this access alone is sufficient for constitutive transcription of *lacZ* promoters. The promoter of the *rosy* gene would also be regulated in a parasegment-specific way by the chromosome state, although there may be additional cell-specific regulatory constraints due to the *rosy* regulatory sequences. GALLONI *et al.* (1993) have also proposed a model of chromatin accessibility to explain the *lacZ* pattern they observe from an enhancer trap in the *iab-7* region. The results described here with a more extensive collection of enhancer traps support such a model.

There are examples of eukaryotic enhancers which lie tens of kilobases from their natural promoters, and which can interact with multiple promoters (KIM *et al.* 1992). It is possible that such enhancers act by looping without the need for a unique target sequence at the promoter. It is also possible that these enhancers affect chromatin accessibility.

**Competition of regulatory signals:** Several enhancer trap lines carry regulatory DNA fragments from the bithorax complex within the  $P$  element, and these sequences direct segment-specific *lacZ* expression when

the  $P$  elements are inserted at other places in the genome. When these elements lie within the complex, there is a potential competition of regulatory information between sequences inside and outside the elements. In four cases ( $Ubx^{Plac(-31)}$ ,  $bxd^{Plac(-31)B}$ ,  $bxd^{Plac(-31)A}$  and  $bxd^{Plac(+13)}$ ), the internal DNA fragments initiate a *lacZ* pattern beginning in PS6, but the *lacZ* pattern from the external regulation overlaps the PS6 boundary. Thus, one cannot tell whether the internal sequences are functional.

In the  $iab-7^{Plac(+132)}$  line, the initiation element inside the  $P$  transposon is activated in PS6–13 (Figure 2A) by gap and pair-rule products, which should block Pc-G repression. But the initiation element(s) outside the  $P$  transposon is repressed by gap and pair-rule genes in parasegments anterior to PS12 (Figure 2E), and this repression would normally be fixed by the Pc-G. In this case, both *lacZ* patterns are superimposed (Figure 2C). Apparently activation prevails over repression, since the final pattern looks like the summation of positive inputs. The prevention of Pc-G repression in PS6–11 might be expected to cause anterior misexpression of the *AbdB* gene. The *Mcp*-like and *Fab*-like phenotypes are indications of such misexpression.

The internal sequences of the  $iab-7^{Plac(+132)}$   $P$  element are not able to maintain the PS6 *lacZ* boundary when this  $P$  element is inserted at other chromosome locations (Figure 2B), but in the *iab-7* location, adjacent BX-C sequences impose strict maintenance of this boundary (Figure 2D). This context-dependent fixation of the PS6 boundary is similar to the results from SIMON *et al.* (1993), where *Polycomb* response elements from one DNA fragment can act on pattern initiation elements on an adjacent DNA fragment.

The other enhancer trap with a potential conflict is the  $iab-8^{Plac(+159)\Delta}$  insertion. This  $P$  element contains DNA from the *iab-3* and *Mcp* regions. The *iab-3* fragment normally programs a pattern starting in PS8

(SIMON *et al.* 1990), but that pattern is not detectable in most embryos that contain this particular construct outside of the BX-C. The *Mcp* DNA fragment, which has been proposed to act as a boundary element (GYURKOVICS *et al.* 1990), may be blocking activation of the *Ubx* promoter by the *iab-3* DNA (see map in Figure 3B). In the double *iab-8* insertion (*iab-8<sup>Plac(+159)</sup>*), the *iab-3* sequences on the distal *P* element could affect the *Ubx* promoter on the proximal *P* element, without an intervening *Mcp* element (Figure 3A). The double insertion shows superimposed patterns from internal and external regulation, with PS8 and PS13 boundaries respectively. It also shows the *Ubx* promoter basal pattern in parasegments 3–7 (Figure 3A), which is not observed in the other enhancer traps within the complex. It is possible that the two *Mcp* boundaries on the construct act to form an isolated domain shielded from outside repression.

**Additional utility of BX-C enhancer traps:** These enhancer traps should be useful for further genetic analysis of the bithorax complex. If the *P* elements are exposed to *P* transposase, they excise with a high frequency, and imprecise excisions generate deletions of adjacent chromosomal sequences (DANIELS *et al.* 1985; GALLONI *et al.* 1993). The enhancer trap elements also allow the possibility of gene conversion in the neighborhood of the insertion site (GLOOR *et al.* 1991). This should permit a detailed analysis of regulatory sequences in the context of the normal complex, or the insertion of more sophisticated probes of chromosome structure.

The parasegment-specific expression from these enhancer traps show which regulatory domain the traps have marked, and this may prove to be the best way to map the extents of the successive domains. The present mapping, based on the phenotypes of a limited number of mutations, is rather crude, especially for the domains of the abdominal region (PS8–14). It may be possible to generate additional enhancer trap lines in the complex by local jumping (ZHANG and SPRADLING 1993) or homing of *P* elements (HAMA *et al.* 1990).

We thank JEFF SIMON, CHRISTIAN KLÄMBT, COREY GOODMAN, LYNNE SCHNEIDER and ALLAN SPRADLING for enhancer trap lines, NORBERT PERRIMON for the BP101 antibody, IAN DUNCAN for UBX antibody, JACK WRIGHT for assistance in generating *Ubx<sup>-</sup>* derivatives of *bx<sup>Plac(-61)</sup>* and SUE CELNIKER for help with the *AbdB* sequence and for ABD antibody. We are grateful to JAY HIRSH and members of the lab for critical reading of the manuscript. This work was supported by grants from the National Institutes of Health to M.O.C. and W.B.

#### LITERATURE CITED

- BEADLE, G. W., and B. EPHRUSSI, 1935 The differentiation of eye pigments in *Drosophila* as studied by transplantation. *Genetics* **21**: 225–247.
- BEACHY, P. A., S. L. HELFAND and D. S. HOGNESS, 1985 Segmental distribution of bithorax complex proteins during *Drosophila* development. *Nature* **313**: 545–551.
- BELLEN, H. J., C. O'KANE, C. WILSON, U. GROSSNIKLAS, R. K. PEARSON *et al.*, 1989 *P* element-mediated enhancer detection: a versatile method to study development in *Drosophila*. *Genes Dev.* **3**: 1288–1300.
- BELLEN, H. J., S. KOOPER, D. D'EVELYN and J. PEARLMAN, 1992 The *Drosophila* Couch potato protein is expressed in nuclei of peripheral neuronal precursors and shows homology to RNA-binding proteins. *Genes Dev.* **6**: 2125–2136.
- BENSON, M., and V. PIRROTTA, 1988 The *Drosophila* zeste protein binds cooperatively to sites in many gene regulatory regions: implications for transvection and gene regulation. *EMBO J.* **7**: 3907–3915.
- BIENZ, M., G. SAARI, G. TREMMEL, J. MÜLLER, B. ZUEST *et al.*, 1988 Differential regulation of *Ultrathorax* in two germ layers of *Drosophila*. *Cell* **53**: 567–576.
- BIER, E., H. VAESSIN, S. SHEPHERD, K. LEE, K. MCCALL *et al.*, 1989 Searching for pattern and mutation in the *Drosophila* genome with a *P-lacZ* vector. *Genes Dev.* **3**: 1273–1287.
- BIER, E., L. Y. JAN and Y. N. JAN, 1990 *rhomboid*, a gene required for dorsoventral axis establishment and peripheral nervous system development in *Drosophila melanogaster*. *Genes Dev.* **4**: 190–203.
- BREEN, T. R., and P. J. HARTE, 1993 *trithorax* regulates multiple homeotic genes in the bithorax and Antennapedia complexes and exerts different tissue-specific, parasegment-specific and promoter-specific effects on each. *Development* **117**: 119–134.
- BUSTURIA, A., and M. BIENZ, 1993 Silencers in *Abdominal-B*, a homeotic *Drosophila* gene. *EMBO J.* **12**: 1415–1425.
- CELNIKER, S. E., D. J. KEELAN and E. B. LEWIS, 1989 The molecular genetics of the bithorax complex of *Drosophila*: characterization of the products of the *Abdominal-B* domain. *Genes Dev.* **3**: 1424–1436.
- CELNIKER, S. E., S. SHARMA, D. J. KEELAN and E. B. LEWIS, 1990 The molecular genetics of the bithorax complex of *Drosophila*: cis-regulation in the *Abdominal-B* domain. *EMBO J.* **9**: 4277–4286.
- COTÉ, B., W. BENDER, D. CURTIS and A. CHOYNICK, 1986 Molecular mapping of the *rosy* locus in *Drosophila melanogaster*. *Genetics* **112**: 769–783.
- CURTIS, D., 1990 Molecular studies on recombination and gene expression at the *rosy* locus in *Drosophila melanogaster*. Ph.D. Thesis, Harvard University.
- DANIELS, S. B., M. MCCARRON, C. LOVE and A. CHOYNICK, 1985 Dysgenesis-induced instability of *rosy* locus transformation in *Drosophila melanogaster*: analysis of excision events and the selective recovery of control element deletions. *Genetics* **109**: 95–117.
- DOYLE, H. J., R. KRAUT and M. LEVINE, 1989 Spatial regulation of *zerknüllt*: a dorsal-ventral patterning gene in *Drosophila*. *Genes Dev.* **3**: 1518–1533.
- DUNCAN, I., 1987 The bithorax complex. *Annu. Rev. Genet.* **21**: 285–319.
- DUNN, T. M., S. HAHN, S. OGDEN and R. F. SCHLEIF, 1984 An operator at -280 base pairs that is required for repression of *araBAD* operon promoter: addition of DNA helical turns between the operator and promoter cyclically hinders repression. *Proc. Nat. Acad. Sci. USA* **81**: 5017–5020.
- EISENBERG, J. C., and S. C. R. ELGIN, 1991 Boundary functions in the control of gene expression. *Trends Genet.* **7**: 335–340.
- ENGSTROM, Y., S. SCHNEUWLY and W. J. GEHRING, 1992 Spatial and temporal expression of an *Antennapedia/lacZ* construct integrated into the endogenous *Antennapedia* gene of *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **201**: 65–80.
- GALLONI, M., H. GYURKOVICS, P. SCHEDL and F. KARCH, 1993 The blue-tail transposon: evidence for independent cis-regulatory domains and domain boundaries in the bithorax complex. *EMBO J.* **12**: 1087–1097.
- GAVIS, E. R., and R. LEHMAN, 1992 Localization of *nanos* RNA controls embryonic polarity. *Cell* **71**: 301–313.
- GLASER, R. G., M. F. WOLFNER and J. T. LIS, 1986 Spatial and temporal pattern of *hsp26* expression during normal development. *EMBO J.* **5**: 747–754.
- GLOOR, G. B., N. A. NASSIF, D. M. JOHNSON-SCHLITZ, C. R. PRESTON and W. R. ENGELS, 1991 Targeted gene replacement in *Drosophila* via *P* element-induced gap repair. *Science* **253**: 1110–1117.
- GYURKOVICS, H., J. GAUSZ, J. KUMMER and F. KARCH, 1990 A new homeotic mutation in the *Drosophila* bithorax complex removes a boundary separating two domains of regulation. *EMBO J.* **9**: 2579–2585.

- HADORN, E., and I. SCHWINCK, 1956 A mutant of *Drosophila* without isoxanthopterin which is non-autonomous for the red eye pigments. *Nature* **177**: 940-941.
- HAMA, C., Z. ALI and T. B. KORNBERG, 1990 Region-specific recombination and expression are directed by portions of the *Drosophila engrailed* promoter. *Genes Dev.* **4**: 1079-1093.
- INGHAM, P. W., 1983 Differential expression of bithorax complex genes in the absence of the *extra sex combs* and *trithorax* genes. *Nature* **306**: 591-593.
- KARCH, F., W. BENDER and B. WEIFFENBACH, 1990 *abdA* expression in *Drosophila* embryos. *Genes Dev.* **4**: 1573-1587.
- KAUFMAN, T. C., R. LEWIS and B. WAKIMOTO, 1980 Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*: the homeotic gene complex in polytene chromosome interval 84A-B. *Genetics* **94**: 115-133.
- KIM, C. G., E. M. EPNER, W. C. FORRESTER and M. GROUNDINE, 1992 Inactivation of the human  $\beta$ -globin gene by targeted insertion into the  $\beta$ -globin locus control region. *Genes Dev.* **6**: 928-938.
- KUZIURA, M. A. and W. MCGINNIS, 1988 Different transcripts of the *Drosophila Abd-B* gene correlate with distinct genetic subfunctions. *EMBO J.* **7**: 3233-3244.
- LEWIS, E. B., 1978 A gene complex controlling segmentation in *Drosophila*. *Nature* **276**: 565-570.
- LINDSLEY, D. L., and G. G. ZIMM, 1992 *The Genome of Drosophila melanogaster*. Academic Press, New York.
- MAZO, A. M., D.-H. HUANG, B. A. MOZER and I. B. DAWID, 1990 The *trithorax* gene, a trans-acting regulator of the bithorax complex in *Drosophila*, encodes a protein with zinc-binding domains. *Proc. Natl. Acad. Sci. USA* **87**: 2112-2116.
- MLODZIK, M., and Y. HIROMI, 1992 Enhancer trap method in *Drosophila*: its application to neurobiology. *Methods Neurosci.* **9**: 397-414.
- MÜLLER, J., and M. BIENZ, 1991 Long range repression conferring boundaries of *Ultrabithorax* expression in the *Drosophila* embryo. *EMBO J.* **10**: 3147-3155.
- MÜLLER, J., and M. BIENZ, 1992 Sharp anterior boundary of homeotic gene expression conferred by the *fushi tarazu* protein. *EMBO J.* **11**: 3653-3661.
- NOSE, A., V. B. MAHAJAN and C. S. GOODMAN, 1992 Connectin: a homophilic cell adhesion molecule expressed on a subset of muscles and the motoneurons that innervate them in *Drosophila*. *Cell* **70**: 553-567.
- OCHMAN, H., A. S. GERBER and D. L. HARTL, 1988 Genetic applications of an inverse polymerase chain reaction. *Genetics* **120**: 621-623.
- O'KANE, C., and W. J. GEHRING, 1987 Detection *in situ* of genomic regulatory elements in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **84**: 9123-9127.
- PARO, R., 1990 Imprinting a determined state into the chromatin of *Drosophila*. *Trends Genet.* **6**: 416-421.
- PEIFER, M., F. KARCH and W. BENDER, 1987 The bithorax complex: control of segmental identity. *Genes Dev.* **1**: 891-898.
- QIAN, S., M. CAPOVILLA and V. PIRROTTA, 1991 The *bx* region enhancer, a distant *cis*-control element of the *Drosophila Ubx* gene and its regulation by *hunchback* and other segmentation genes. *EMBO J.* **10**: 1415-1425.
- ROBERTSON, H. M., C. R. PRESTON, R. W. PHILLIS, D. M. JOHNSON-SCHLITZ, W. K. BENZ *et al.*, 1988 A stable genomic source of *P* element transposase in *Drosophila melanogaster*. *Genetics* **118**: 461-470.
- SAARI, G., and M. BIENZ, 1987 The structure of the *Ultrabithorax* promoter of *Drosophila melanogaster*. *EMBO J.* **6**: 1775-1777.
- SADOWSKI, I., J. MA, S. TRIEZENBERG and M. PTASHNE, 1988 GAL4-VP16 is an unusually potent transcriptional activator. *Nature* **335**: 563-564.
- SANCHEZ-HERRERO, E., and M. A. CROSBY, 1988 The *Abdominal-B* gene of *Drosophila melanogaster*: overlapping transcripts exhibit two different spatial distributions. *EMBO J.* **7**: 2163-2173.
- SHIMELL, M. J., J. SIMON, W. BENDER and M. B. O'CONNOR, 1994 Enhancer point mutation results in a homeotic transformation in *Drosophila*. *Science* **264**: 968-971.
- SIMON, J., M. PEIFER, W. BENDER and M. O'CONNOR, 1990 Regulatory elements of the bithorax complex that control expression along the anterior-posterior axis. *EMBO J.* **9**: 3945-3956.
- SIMON, J., A. CHIANG and W. BENDER, 1992 Ten different *Polycomb* group genes are required for spatial control of the *abdA* and *AbdB* homeotic products. *Development* **114**: 493-505.
- SIMON, J., A. CHIANG, W. BENDER, M. J. SHIMELL and M. O'CONNOR, 1993 Elements of the *Drosophila* bithorax complex that mediate repression by *Polycomb* group products. *Dev. Biol.* **158**: 131-144.
- STRUHL, G., and M. AKAM, 1985 Altered distributions of *Ultrabithorax* transcripts in *extra sex combs* mutant embryos of *Drosophila*. *EMBO J.* **4**: 3259-3264.
- WHITE, R. A. H., and M. WILCOX, 1984 Protein products of the bithorax complex in *Drosophila*. *Cell* **39**, 163-171.
- WHITE, R. A. H., and M. WILCOX, 1985 Regulation of the distribution of *Ultrabithorax* proteins in *Drosophila*. *Nature* **318**: 563-567.
- WILSON, C., R. K. PEARSON, H. J. BELLEN, C. J. O'KANE, U. GROSSNIKLUS *et al.*, 1989 *P* element-mediated enhancer detection: an efficient method for isolating and characterizing developmentally regulated genes in *Drosophila*. *Genes Dev.* **3**: 1301-1313.
- ZAVORTINK, M., and S. SAKONJU, 1989 The morphogenetic and regulatory functions of the *Drosophila Abdominal-B* gene are encoded in overlapping RNAs transcribed from separate promoters. *Genes Dev.* **3**: 1969-1981.
- ZHANG, P., and A. C. SPRADLING, 1993 Efficient and dispersed local *P* element transposition from *Drosophila* females. *Genetics* **133**: 361-373.
- ZINK, B., and R. PARO, 1989 *In vivo* binding of a trans-regulator of homeotic genes in *Drosophila melanogaster*. *Nature* **337**: 468-471.

Communicating editor: R. E. DENELL